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LEUKEMIA OF THE COMMON FOWL.*

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LEUKEMIA appears to be of rather frequent occurrence in the domestic animals. The statistics of Nocard (1880-82) comprised twenty-two cases in the dog, nine in the horse, six in the hog, five in the cow, and one in the cat. Since 1891 a number of new cases of leukemia in cattle, horses, dogs, and swine have been observed, and the disease has been shown to occur also, though less frequently, in the goat, sheep, and mouse. In the case of the rabbit and guinea-pig, the animals most frequently used in experimental work, there exist at present no observations of the occurrence of leukemia. The literature is likewise devoid of any statements concerning its occurrence in birds and fowls.

Moore, in 1895-96, reported observations upon an epidemic disease of fowls under the designation of "infectious leukemia." From the blood of diseased fowls showing a great increase in the total number of white cells he isolated *B. sanguinarium*, and regarded this as the etiological agent. There can be no doubt that the condition described by Moore was not one of true leukemia, but only a marked leukocytosis due to a bacillary infection. The increase in the white cells appears from his description to have been limited to the crystalloid eosinophile cells. No atypical mononuclear cells are mentioned, and the general pathological picture is that of an infectious disease and not that of leukemia. The cellular infiltrations of spleen and liver so characteristic of the leukemias were wanting, and the very short course of the process speaks for the non-leukemic nature of the infection. Moore's cases must, I think, be rejected as instances of avian leukemia.

Butterfield, in 1905, reported from this laboratory three cases of aleukemic lymphadenoid tumors of the hen. In all three cases attention had been directed to the very marked enlargement of the liver, the material having been sent to me for examination because of this feature. The histological examination of the livers of the three cases showed the same condition in all. The liver tissue was nearly wholly

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replaced by masses of cells resembling in general the large lymphocytes of the hen's blood, although showing slight individual variations. With the exception of slight changes due to pressure, these cells were round or oval, and possessed round or oval nuclei, usually placed somewhat eccentrically. The chromatin was reticular or granular, and the narrow rim of protoplasm nongranular and basophilic. Between the cells there was a delicate stroma. Evidences of rapid growth were found in the presence of numerous mitoses. The small amount of liver tissue remaining consisted of strands or islands of liver cells lying in or between the masses of lymphoid cells, the liver cells showing the effects of compression conforming to the shape of the tumor areas. The latter were oriented as being periportal, the smaller groups of tumor cells surrounded by hepatic cells as being direct outgrowths of the periportal masses in the great majority of instances, although apparently metastatic in others. At the borders of the tumor masses great numbers of crystalloid eosinophiles, mostly mononuclears, were seen. No increase of white cells was found in the blood-vessels. The type of cell, the periportal growth of the tumor masses, and the absence of any increase of white cells in the circulation led to the diagnosis of *aleukemic lymphadenoma*, or, according to the terminology proposed by me as *aleukemic leukoblastoma* of the lymphocyte type (*lymphocytoma*).

The establishment of the existence of an aleukemic lymphocytoma in the fowl led me to believe that the leukemic form must also exist and that its demonstration was only a question of chance and material. Arrangements were made by which fowls showing symptoms of disease other than the common poultry affections would be brought to the laboratory for examination. Dr. Butterfield in the meantime, had learned through correspondence with Dr. Mohler of the Bureau of Animal Industry that the latter had examined the carcasses of five fowls showing a condition of disseminated lymphadenoma with an increase of the lymphocytes in the blood-vessels. Through the courtesy of Dr. Mohler slides were sent to us for examination, and from these there could be no doubt that the condition was one of leukemic lymphocytoma. The tumor-like infiltrations of cells presented the same histological features as in our cases, but in addition the blood-vessels contained great

numbers of white cells, the predominating type being large lymphocytes precisely like the cells of the tumor-like infiltrations in both his cases and ours. As Dr. Mohler had unfortunately examined only the carcasses, no blood-counts or differential counts in stained smears were made, and for this reason these cases were not reported, Dr. Mohler awaiting an opportunity of studying the condition in the living fowl. To my knowledge this has not yet been done; but an opportunity is here taken of crediting Dr. Mohler with the observations made by him.

In December, 1905, there came into my hands a Buff Cochins Bantam hen showing signs of illness in the way of indisposition to move about and a general weakness of a progressive character. No symptoms of the ordinary fowl diseases were present. Catarrhal conditions were entirely absent. As a number of fowls presenting similar symptoms had been examined and found to have avian tuberculosis, it was thought at first sight that this fowl was similarly affected. Examination of blood-smears showed, however, a great increase of white cells of the large lymphocyte type, instead of the crystalloid eosinophile leukocytosis found in the tuberculous hens. A diagnosis of leukemia was therefore made, and thorough blood-examinations were carried out until the death of the fowl, which took place about two and one-half months after it was first seen. During this time the hen showed a progressive weakness and emaciation, with periods of apparent improvement. The comb lost its red color, becoming gray, the yellow portions of the legs also became gray. Much of the time the hen maintained a sitting posture, and when disturbed would move a few steps and then sit down. Food was taken in very small amounts except during the periods of apparent improvement. For a few days before death thin watery fecal discharges were noted. Death resulted from increasing weakness, the fowl dying while a blood-count was being taken.

BLOOD.

The blood for counting and for smears was obtained from cuts made into the comb with a sharp knife. The slight wounds thus made bled abundantly. Toison's solution was found to be the most satisfactory for the blood-counting. Hematoxylin and eosin, Ehrlich's triple stain and Wright's method were used for staining the smears. The best results, on the whole, were obtained by Wright's, when this stain was used immediately after the smears were made. When used with smears that had been

allowed to dry in the air for some time before staining, both red and white cells showed marked degeneration and stained poorly.

Blood-count.—Red-blood cells=450,000; white cells=280,000. This gives a proportion of less than two red cells to a white cell. While considerable variation in the number of white and red cells was found at different times, the above represents the average count.

Stained smears.—These showed the great increase of white cells found by the blood-count, and numerous differential counts were made. While showing some variation in the smears made at different times the average differential count, 500 white cells, was as follows:

Small lymphocytes	1.5 per cent
Large lymphocytes	84.5 "
Granular eosinophiles	0.0 "
Crystalloid eosinophiles	11.5 "
Degenerating white cells	2.0 "
Mast cells	0.5 "
	<hr/>
	100.0 "

In some counts the proportion of large lymphocytes was found as high as 90 per cent, in others as low as 80 per cent. These cells varied greatly in size, all transition-stages being found between those of normal size and those several times as large. The protoplasm was basophilic and usually non-granular, and varied greatly in amount, from a small rim around the nucleus to a band exceeding in width the diameter of the nucleus. The latter stained a clear light blue in the smaller forms of the large lymphocytes, in the large ones a decided reddish violet. In the majority of cells the nucleus was placed somewhat eccentrically. It was reticular or granular, and in many cells vacuolated. In the larger cells with nuclei staining violet, the chromatin was much more reticular than in the smaller cells. These cells also contained more and larger vacuoles, and are probably to be regarded as degenerating lymphocytes. No evidence of any intra-cellular parasite or inclusion could be found in the lymphocytes. No division figures were found. The small lymphocytes were greatly reduced in number, as were also the eosinophile cells. Of the latter, numerous mononuclear forms were found. The mast cells were not increased. The red-blood cells were pale, and showed marked poikilocytosis. The labile cells were diminished in number, and many atypical forms of these were seen, some containing two nuclei.

As a control, counts and stained smears were made of the blood from healthy fowls of the same variety. Considerable variation was found in the number of both red and white cells under apparently normal conditions. The average count may be given as follows:

Normal Hen's (Buff Cochin Bantam) red cells=2,000,000-3,000,000.
 " " " " " white cells=12,000-25,000.

A proportion of 105-225 red cells to one white cell obtains.

DIFFERENTIAL COUNT.

Small lymphocytes	35.5 per cent
Large "	14.5 "
Granular eosinophiles	10.0 "
Crystalloid "	21.5 "
Degenerates	16.5 "
Mast cells	2.0 "
	<hr/>
	100.0 "

When compared with the blood of normal hens of the same variety the blood of the diseased fowl presented the following differences:

1. Great reduction in the total number of red-blood cells, only one-sixth to one-eighth of the normal number being present. These showed great variation in size and shape, and the haemoglobin was much reduced. The labile cells were diminished in number and evidences of nuclear degeneration were found in many of them.

2. A great increase in the total number of white cells, so that a proportion of one white cell to two reds obtained. Further, a marked disturbance in the relative proportion of the different types of white cells, the polymorphonuclear cells and small lymphocytes being replaced to a large extent by cells of the large lymphocyte type. Numerous atypical mononuclear leukocytes were also present in the blood.

From the blood-changes a diagnosis of *lymphatic leukemia* of the large-cell type seemed justified and was accordingly made.

AUTOPSY PROTOCOL.

The autopsy of the fowl was made immediately after its death, while the tissues were still warm.

Small, emaciated fowl. Total weight 450 grms. Comb and wattles very anemic, grayish in color. No fat in body. Small amount of pale blood present in heart and blood-vessels. All tissues pale and anemic. Muscles very atrophic.

Liver.—Weight 34 grms., about $\frac{1}{8}$ th of the entire body weight. Greatly increased in size: five or six times that of the normal Bantam liver of same variety. It filled up the greater part of the abdominal cavity. The general shape was fairly well preserved; somewhat more spherical than normal. Color was pale yellow; uniform surface; consistency soft, crumbling, very easily torn. No liver structure was apparent. Scrapings yielded abundant cells of type of large lymphocyte, in all respects identical with those of the circulating blood.

Spleen.—Enlarged; spherical; Weight 2.5 grms; reddish-yellow in color; soft and friable. On section, presented numerous yellowish spots about the size of a pinhead.

Heart.—Weight 3.5 grms; dilated; muscle pale, thin, and soft. Contained a few small yellowish points in the muscle.

Kidneys.—Weight 2.0 grms. each; enlarged. Throughout the kidney substance there were numerous small, yellowish spots, varying in size from that of a pin-point to that of a mustard-seed.

Hemolymph Nodes.—Two large reddish-yellow masses lay along the median line of the backbone. Weight 2.5 grms. each. They also contained small yellowish nodules.

Bone-marrow.—From the femurs solid plugs of reddish-yellow, friable marrow were obtained.

With the exception of atrophy and anemia the other organs and tissues presented no pathological appearances.

MICROSCOPICAL EXAMINATION.

Preparation.—Smears were made of the blood and from the cut surfaces of all the organs and tissues. Part of these were fixed in alcohol and ether and stained with hematoxylin and eosin, Ehrlich's triple stain, etc. The others were stained by Wright's method. Portions of the organs and tissues were fixed in mercuric chloride, Zenker's, 4 per cent formaldehyde, and Flemming's solution. They were imbedded in paraffin and stained in various ways.

Smears.—The smears of blood taken at the autopsy resembled those made during life, with the exception that the number of degenerating white cells was greatly increased. The smears made from the liver, spleen, and bone-marrow showed a preponderance of cells of the large lymphocyte type, identical with those in the circulating blood.

Liver.—Sections of this organ showed the liver-tissue to be almost entirely replaced

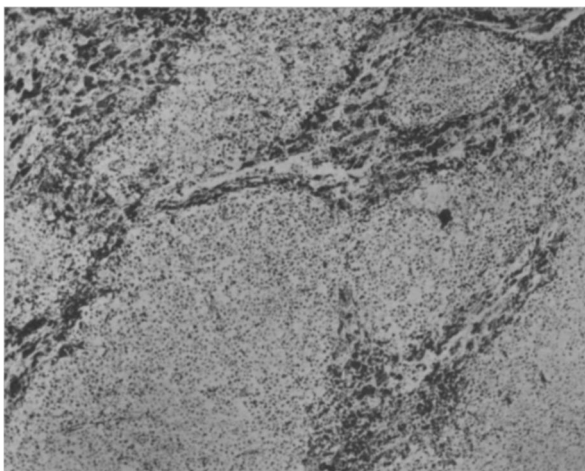


FIG. 1.—Fowl's liver in leukemia. Cells of large lymphocyte type arranged in periportal tumor-like masses infiltrating and destroying the liver-tissue. Leukemic infiltration.

in the circulating blood. Between these cell-masses there were present strands and islands of liver cells in varying stages of atrophy, cloudy swelling, and fatty degeneration. The capillaries between the liver-rods were filled with white cells, in great excess over the red cells. As no portal structures were found in the areas of hepatic tissue, and as these could occasionally be seen in the masses of lymphoid cells, the latter were oriented as periportal infiltrations and proliferations. Smaller islands of lymphoid cells apparently surrounded by hepatic cells were found to be continuous with each other and with the larger masses. The appearance of multiple tumor-nodules shown by a single section is explained by serial sections as resulting from such outgrowths into the lobules from the main periportal masses. Staining with Mallory's reticulum stain showed that each large nodule had for its center a periportal tract.

The cells of the periportal infiltrations showed slight individual variation. They were round or oval, and as a rule possessed pale, round, or oval nuclei, placed somewhat eccentrically. The chromatin was granular or reticular. Vesicular nuclei with deeply staining nucleoli were often seen. The basophilic protoplasm formed a ring of varying width about the nuclei. Numerous mitotic figures, apparently nor-

mal, were seen throughout the cell masses. No multinuclear or giant cells were seen. Between the cells there could be demonstrated a delicate stroma. No retrograde changes were found in any of the areas of lymphoid cells. In some nodules the lymphocytes were packed more densely around the periphery, giving an appearance resembling that of a germ-center. In the peripheral zone of the lymphoid collections bordering upon the

hepatic cells great numbers of crystalloid eosinophiles were seen, the majority of them being mononuclears. The larger blood-vessels of the liver showed an excess of white cells over red, the great majority of the former corresponding to the cells of the

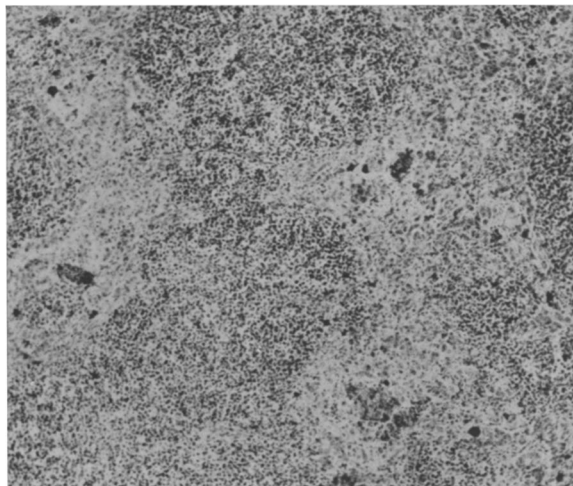


FIG. 2.—Fowl's liver in leukemia. Parenchyma nearly wholly replaced by lymphoid growths.

periportal masses and to the type of the large lymphocytes of the blood-smears.

Spleen.—Sections of the spleen showed a marked hyperplasia of the stroma and a replacement of normal spleen tissue by an atypical lymphoid tissue similar to that found in the liver. The yellowish points consisted of collections of lymphocytes apparently in more rapid proliferation, the cells being crowded densely around the periphery of a lighter-staining

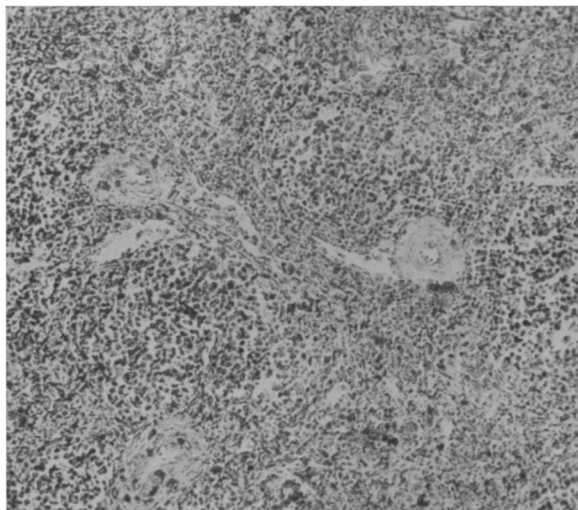


FIG. 3.—Fowl's spleen in leukemia. Normal spleen tissue replaced by cells of large lymphocyte type, sometimes arranged in masses suggesting centers of proliferation. The stroma is also increased.

central area. Eosinophiles were not so numerous as in the liver, and had no definite arrangement. The same excess of white cells was found in the larger vessels as that seen in the hepatic blood-vessels.

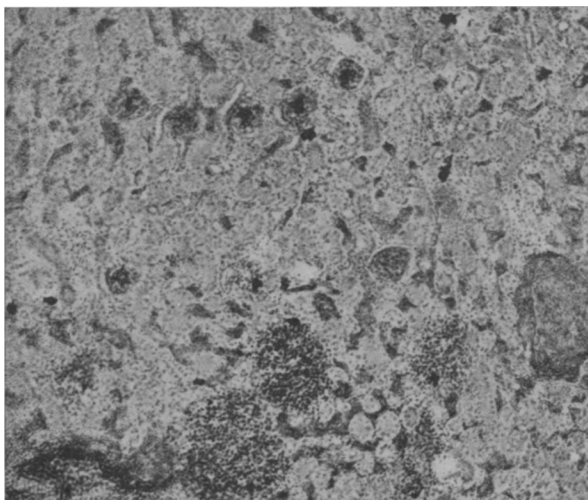


FIG. 4.—Kidney of leukemic fowl. The glomeruli show various stages of lymphocyte embolism and proliferation interpreted as leukemic metastases.

blood and in the infiltrations in the liver and spleen. These nodules occupied the sites of glomeruli, and all transition stages of development could be seen, from glomeruli containing a small collection of these cells in the glomerular capillaries, suggesting an embolus, to larger masses completely destroying and replacing the glomeruli. About the larger nodules the kidney structures were compressed or destroyed, and cords of lymphoid cells infiltrated out from the main mass into the surrounding renal tissue, in all respects after the manner of

Heart.—Sections of this organ showed an excess of white cells of the large lymphocyte type in the blood-vessels, and a few small perivascular infiltrations of cells of the same type as those in the liver and spleen. The muscle was atrophic and showed slight fatty degeneration.

Kidneys.—Throughout both kidneys numerous small nodules of lymphoid cells occurred, varying greatly in size, but made up of the same type of large lymphocyte found in the

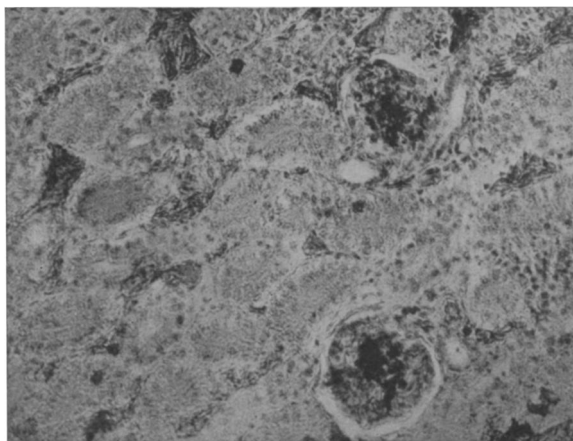


FIG. 5.—High-power view of preceding showing collections of lymphocytes in glomerular capillaries.

a malignant tumor. Not all glomeruli contained cell-masses; some were entirely free from any lymphocyte collections. Others showed only an excess of white cells in the capillaries without any apparent embolism or any proliferation. On the whole the appearances give the impression that the nodules of lymphoid cells arising in the glomeruli are of the nature of tumor-metastases arising from emboli of lymphoid cells in the glomerular capillaries. Mitotic figures were common in these nodules. Their infiltrative and destructive action upon the surrounding kidney structures is in all ways analogous to that seen in the case of malignant tumors. The blood-vessels of the kidney showed a great excess of white cells over red.

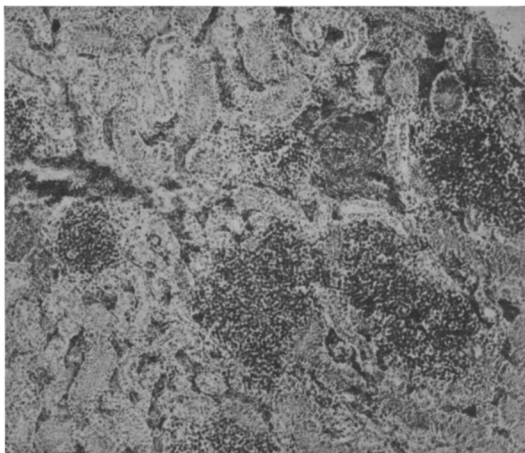


FIG. 6.—Leukemic metastases and infiltration of fowl's kidney.

Bone-marrow.—The bone-marrow appeared to consist chiefly of cells of the large

lymphocyte type, the number of red-blood cells, eosinophiles, and mast cells being greatly diminished. No giant or multinuclear cells were found. Throughout the marrow there were numerous small areas having a lighter center and a densely crowded periphery, resembling germ-centers. The cells composing these areas were of the large lymphocyte type, similar in all respects to the cells of the tumor-nodules and infiltrations in

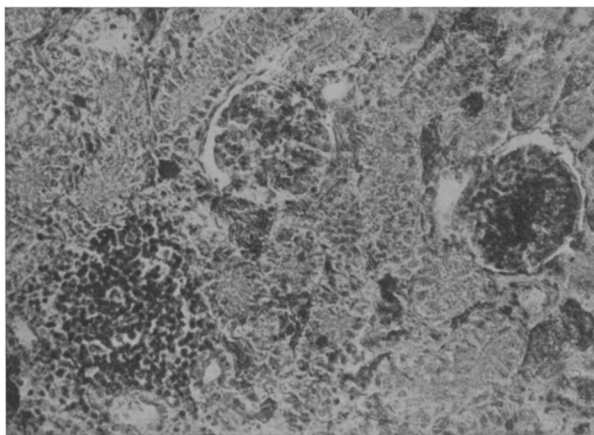


FIG. 7.—Kidney of leukemic fowl. Three glomeruli in various stages of lymphocyte infiltration. One glomerulus is practically normal, a second one contains a lymphocyte embolus with beginning proliferation, while in the third the growth of the lymphocytes has destroyed the glomerulus and there is an initial infiltration of the surrounding renal tissue.

the liver, spleen, and kidneys. The changes in the marrow were interpreted as being of the nature of a lymphoid hyperplasia replacing the red-cell-forming tissue. The

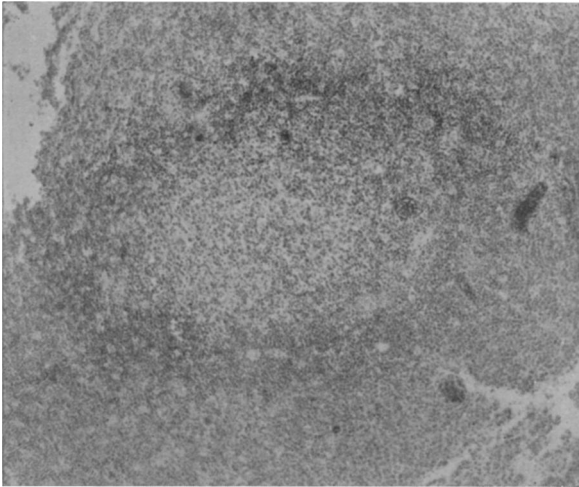


Fig. 8.— Bone-marrow of leukemic fowl. The light area with darker border represents either a primary center of lymphocyte proliferation or may be interpreted as a metastasis.

areas resembling germ-centers might be explained as metastases or as centers of more active proliferation.

Hemolymph nodes.—The reddish masses lying along the backbone were shown microscopically to be made up of collections of lymphoid cells and blood-sinuses. The type of cell was that found elsewhere, as described above. The blood-vessels and sinuses contained a great excess of white cells of the large lymphocyte type.

The examination of the remaining organs and tissues showed throughout the leukemic blood-picture in all the blood-vessels with the occurrence of extra-vascular collections of lymphocytes. The parenchyma of all the organs exhibited more or less atrophy and fatty change.

The tissue changes may, then, be summarized as follows:

1. Tumor-like nodules and infiltration of lymphoid cells in all the organs, particularly in the liver, spleen, kidneys, bone-marrow, and hemolymph nodes.
2. Replacement of the normal white cells of the blood by cells of large lymphocyte type, more or less atypical in character but identical with the cells of the tumor-masses.
3. Secondary atrophy and degeneration.

DIAGNOSIS.

The findings, both in the blood and in the tissues, fully warrant the diagnosis of *leukemic lymphocytoma* (large-celled lymphemia).

ETIOLOGY.

All attempts to discover an etiological agent failed. The examina-

tion of the blood and tissues for bacterial and protozoan parasites was thoroughly carried out and gave absolutely negative results. A great variety of staining methods were used, including the most recent methods for the staining of spirochetes and protozoan parasites. Nothing suggesting a parasite was found. No inclusions of any kind were seen in the white cells. Cultures upon a variety of media were sterile, both aerobic and anaerobic. No evidence of the existence of any infective agent could be obtained. The plans for inoculation and transplantation miscarried in this case. They are being prosecuted in the aleukemic variety of lymphocytoma as new material is obtained. It may be mentioned in this connection that Weil and Clerc failed to obtain any results from the inoculation of blood and the transplantation of portions of lymphadenoid tumors in the case of leukemia of the dog.

Since the above case was studied two new cases of lymphadenoid tumors in the common fowl have been received at the laboratory, the material having been sent in by the health officer of Ann Arbor, Dr. Wessinger, for examination for suspected tuberculosis. One of these cases is of the aleukemic condition, as described by Dr. Butterfield; the other is of the leukemic form, as described above. The histological picture apart from the increase of white cells in the blood vessels in the one case, is identical in the two cases and also with that in the cases given above. From these cases we must conclude, therefore, that there occurs in the common fowl a condition of disseminated lymphocytoma or lymphosarcoma, sometimes aleukemic, at other times associated with an increase of the white cells of the blood, this increase consisting in the appearance in great numbers of cells of the large lymphocyte type and apparently identical with the lymphoid cells of the tumor-nodules.

The aleukemic and leukemic conditions must, therefore, be regarded as genetically related, if not actually one and the same process, in different stages. The same thing is true of the leukemic and aleukemic lymphomata or lympho-sarcomata found in man and observed also in the dog; and occurring probably in all other animals in which leukemia has been discovered. The wide-spread occurrence of these affections may be taken as throwing some light upon the nature of the leukemic process. In this respect it resembles the malignant

tumors, these also showing a wide distribution throughout the lower animals. Such a wide distribution speaks against the existence of any specific parasite. In other respects, too, these conditions, both the leukemic and aleukemic, are comparable to malignant tumors. The formation of metastases, the infiltrative and destructive growth, the failure of inoculations and transplantations, etc., all favor the view that they are neoplasms, and present the same problems as do the malignant tumors.

If to one laboratory in the course of a few years there come four cases of aleukemic lymphcytoma and two of leukemic in the common fowl, its occurrence in this animal cannot be so very rare. The opportunity should, therefore, be taken, whenever possible, of making careful comparative pathological studies of these conditions with the view of throwing some light upon the problems of their occurrence in man. As interesting a field is opened here as in the case of carcinoma of the lower animals.

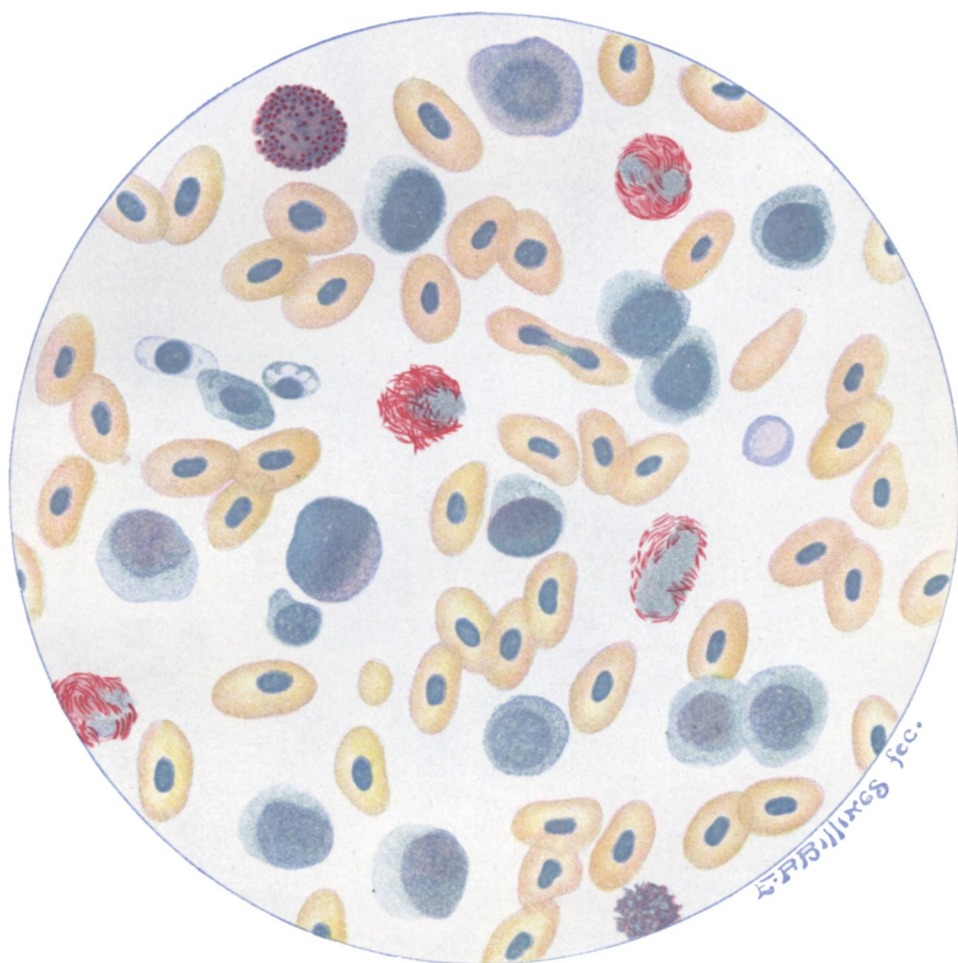
CONCLUSIONS.

1. Aleukemic and leukemic forms of lymphcytoma exist not infrequently in the common fowl, in all respects analogous to the similar conditions existing in man.
2. As in man, the two conditions appear to be genetically related, or different stages of the same process.
3. There is no evidence of an infective causal agent.
4. The essential nature of the two conditions would lead to their interpretation as malignant neoplasms. The problem of leukemia, then, becomes identical with that of malignant neoplasms in general.

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PLATE 14.



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PLATE 14.

Leukemia of the Fowl.